HYPERBRANCHED POLYMERS AND HAAG TYPE DENDRIMERS FOR THE MOLECULAR IMPRINTING OF POLYMERS

BY

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THESIS

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ABSTRACT

A molecularly imprinted polymer was synthesized using both hyperbranched polyglycerol and Haag type polyether dendrons with estradiol as a core. The attachment of the polyether dendron created a dendrimer that had 64 allyl groups available for crosslinking. Completion of the crosslinking of the dendrimer resulted in a polymer that was not fully crosslinked, therefore the polymer was unable to hold its shape and not be effective as a molecular imprint.

ACKNOWLEDGEMENTS

I would first like to thank my advisor, Steven C. Zimmerman, for all of the helpful guidance, advice and encouragement throughout my graduate career. I would also like to thank all of the people who have helped me along the way, including members of the Zimmerman group, especially Dr. Jordan Quinn, who encouraged me to keep going that first year. Finally, I would like to thank my friends and family for all of their support, especially my wife Brittany who was always there for me no matter what the circumstance.

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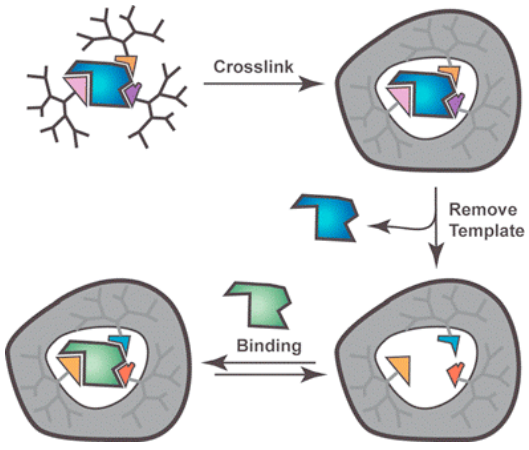
CHAPTER 1:
MOLECULARLY IMPRINTED POLYMERS (MIP)

1.1 Introduction

For the past thirty years, scientists have been attempting to design a synthetic system where a host binds its substrate with a high degree of selectivity. In nature, enzymes do this quite efficiently and will usually only bind a narrow range of substrates. In 1972, Wulff demonstrated[1] that a polymer could mimic enzymes and resolve racemates. Wulff took a modified boronic ester of D-glyceric acid and copolymerized it with divinylbenzene. Upon cleavage of the ester bond, it was found that if a racemic mixture of gylceric acid was introduced into the polymer, the D-gylceric acid was taken into the polymer, leaving the L-gylceric acid still in solution.

Attempts to mimic an enzyme, termed molecular imprinting, has improved over the past few years.[2-4] In this process, a template is enveloped in a polymer containing polymerizable groups such as alkenes. This polymer is crosslinked, and the template is removed (cored) to create a molecularly imprinted polymer (MIP) as seen in Scheme 1. Many different types of polymers are used, including a unique, branched type of polymer our group[5] has used, called a dendrimer.

Scheme 1



Dendrimers are polymeric structures that are different from other polymers in that they are monodisperse and possess a branched structure. They are synthesized through iterative steps that involve attaching subunits together to make a larger unit. Dendrimers are superior over other polymers for molecular imprinting because of their ease of characterization and quantitative template removal.[6]

The synthesis of dendrimers for use in molecularly imprinted polymers can be done either divergently or convergently. In the divergent manner, the repeated units are attached to the template and the dendrimer is grown from it. In the convergent manner, each dendrimer is produced separately and then in the final step they are attached to the template covalently. An advantage of growing these dendrimers is that if one strategy fails, the other strategy may be employed.

There are many templates that can be chosen to imprint. For instance, our group has had success with using porphyrins as templates.[5] Using a Fréchet[7] type dendrimer our group was able to attach dendron **2** to octahydroxyl porphyrin **3** using 1,3-dicyclohexylcarbodiimide (DCC) coupling catalyzed by para-toluenesulfonic acid (Figure 2). This coupling was successful, and product **1** was easily characterized and

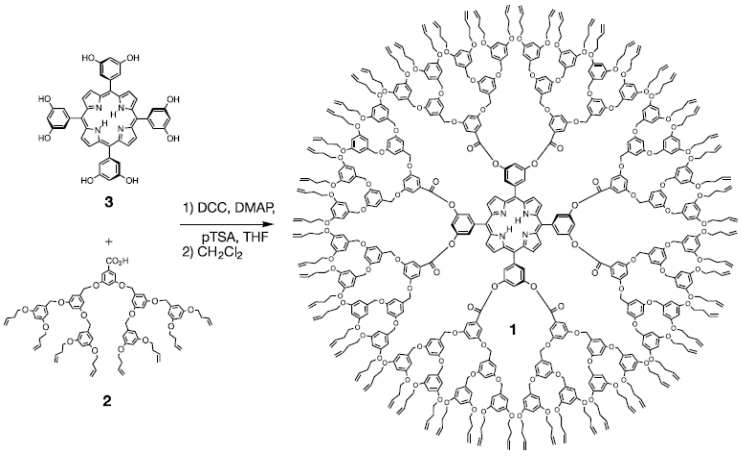


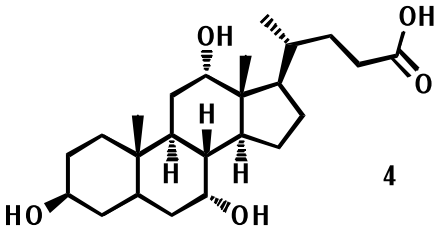
Figure 1. Fréchet type imprinted polymer

found to be pure. Grubbs' catalyst[8] crosslinked the double bonds of the homoallyl groups, creating a rigid structure. Coring the porphyrin by hydrolyzing the ester bonds created an imprinted polymer. This MIP bound similar porphyrins that contained four or more hydrogen bonding sites.

Although this method worked well, there were some things that could be improved. For instance, the aryl groups in the Fréchet dendron made it difficult for monitoring binding of other templates by ultraviolet spectroscopy. Also, the synthesis of the materials was rather tedious. It requires a rather lengthy dendrimer purification process.

1.2 Selection and Synthesis of a Template

An ideal template should be fairly large in size and contain functional groups that can serve as points of attachment to the dendrons. Ideally, the template would have these functional groups evenly spaced throughout the molecule so a large number of dendrons could surround the template. Our group[5] has used porphyrins as templates for their large size and their aromatic phenol groups for easy attachment through an ester bond. Although porphyrins are very good candidates, a porphyrin has very little practical use. A literature search found steroids would be a better candidate as a template for molecularly imprinted polymers.



4

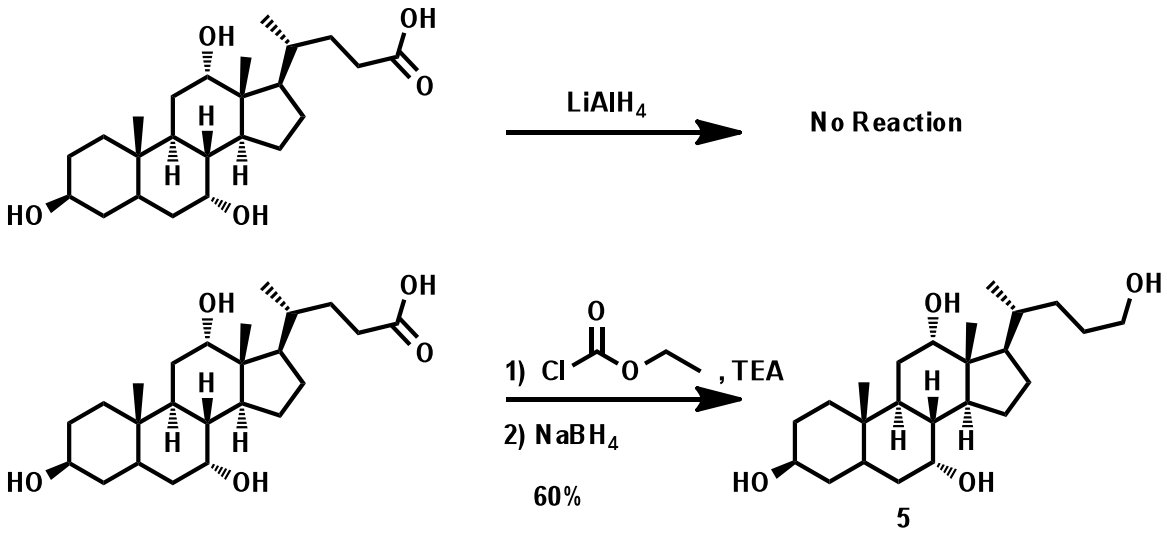
Cholic Acid

Steroids are naturally occurring carbocycles of great importance in biochemistry. They make up a large number of hormones and other substances in the human body responsible for many daily activities. Upon a survey of many steroids[9], cholic acid **4** was seen as a potential candidate. Although it does not have

chromophores that can be seen by ultraviolet light, it does have three hydroxyl groups that allow attachment of dendrimers. Additionally, the acid can be reduced to create a fourth attachment. The binding of the steroid after coring can be determined either by using circular dichroism or optical rotation using a polarimeter.

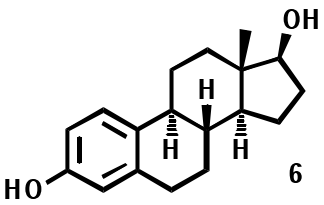
Synthesis of the reduced acid is outlined in Scheme 2. Cholic acid **4** was readily available in our inventory. Initial attempts to reduce the acid using lithium aluminum hydride in tetrahydrofuran were unsuccessful. Alternative methods were sought, and a successful reaction was found.[10] Activation of the acid by treating with ethylchloroformate in triethylamine created the ester in quantitative yield. Subsequent reduction with sodium borohydride afforded **5** in 60 % yield. It was found to be pure by ^{1}H NMR, mass spectroscopy, and by a literature melting point of 215-220°C. Steroid **5** had the potential to be used as the template for MIP synthesis. However, after many attempts of attaching the dendron using esterification, it was not successful. This will be discussed in the next chapter. Also, it was noticed that all of the hydroxyl groups would point in the same direction, not allowing for the dendrons to totally surround the core.

Scheme 2



It was then decided that the core be switched to something else because of the lack of success. Many other organic molecules were surveyed and it was decided that a chloramphenicol core should be used. Chloramphenicol is an oral antibiotic that is used to treat many infections including typhoid fever, so it was envisioned to be a potentially useful core in a molecular imprinting system. After envisioning a synthesis of a chloramphenicol like core and starting the first few steps, one of the transformations was unsuccessful and the core was abandoned.

The core that was selected was another steroid similar to cholic acid, estradiol. Estradiol is an estrogen, which is a naturally occurring female sex hormone. Even though estradiol has two hydroxyl groups compared to the four of the reduced cholic acid, it was deemed efficient to prove the concept of the molecular imprinting. It also has a benzene moiety, so binding could potentially be measured using UV or fluorescence spectroscopy. The estradiol molecule was modified in order to allow attachment of the dendrons and this modification will be shown in detail in the next chapter.



Estradiol

CHAPTER 2:
HAAG TYPE DENDRIMERS

2.1 Introduction

Many dendritic molecules were surveyed. A dendrimer synthesis must be efficient, the yields of the reactions must be high, and purification should be facile. The Haag[11] type dendrimer accomplishes both of these aspects. The Haag dendrimer is a polyether dendrimer that does not contain any aryl groups. It has the advantage that it can be hydrophobic or hydrophilic depending if it contains the allyl moieties or the hydroxyl moeities (Figure 2).

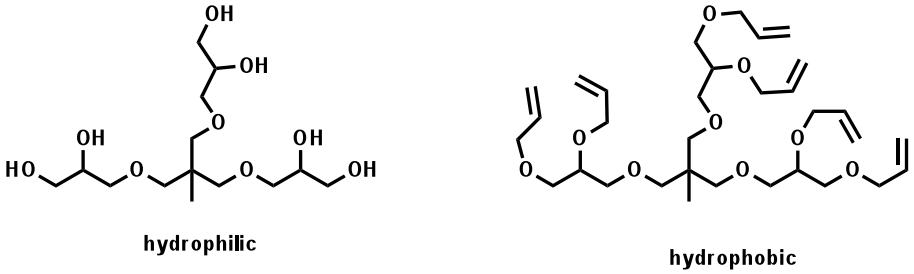


Figure 2. The dual nature of the Haag dendrimer

The general procedure is a Williamson etherification of an alcohol (or polyol) with allyl bromide, followed by catalytic dihydroxylation using potassium osmate dihydrate with N-methyl-morpholine (NMO) as a co-oxidant. When these two steps are repeated, a polyether dendrimer is formed. After allylation, the dendrimer is hydrophobic, and after oxidation of the double bonds, the dendrimer becomes hydrophilic. This is advantageous because the properties of the imprinted system can be changed in one step.

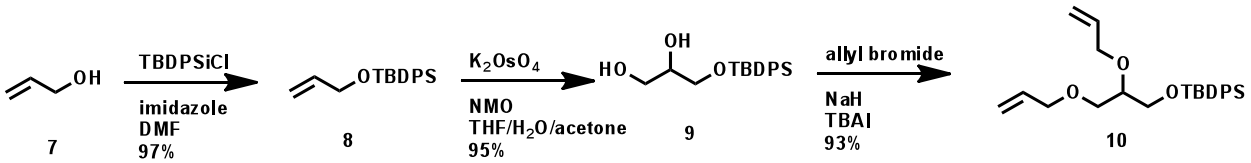
In order to attach the dendrimer on an appropriate template, a synthetic handle must be placed on the dendrimer. One of the most versatile functional groups is the alcohol group, which can be oxidized to the corresponding aldehyde or acid, etherified to an alkyne for a click reaction, or transformed in more complex reactions to produce azides, amines, or many other

functional groups. A suitable protecting group must be used to mask this functionality for later use. Masking is done most efficiently by protecting the alcohol with a tert-butyldiphenylsilyl group. This group is stable to both the etherification and dihydroxylation conditions. Furthermore, the protecting group contains a chromophore that can be visualized by an ultraviolet lamp, allowing for simpler purification on a quartz silica gel column after the allylation steps.

2.2 Synthesis of Dendron

Starting with commercially available allyl alcohol **7**, the synthesis of the dendrimer proceeded as outlined in Scheme 3. Protection of allyl alcohol with tert-butyldiphenylsilyl chloride with imidazole in dimethylformamide afforded compound **8** in 97% yield.[12] **7** was not purified and taken to the next step. Subsequent dihydroxylation afforded **9**; on extraction with ethyl acetate it afforded a tan solid. Precipitation into hexanes afforded a white solid. The literature suggested using a 4 wt % solution of osmium tetroxide in water for the source of the osmium, but the potassium dihydrate salt gave much better results. The removal of the osmium from the reaction mixture was done successfully by rotary evaporating the solvent mixture under reduced pressure. A black solid formed on the top of the round bottom flask; this was assumed to be osmium dioxide. NMO was not present because it is readily soluble in water and removed upon extraction.

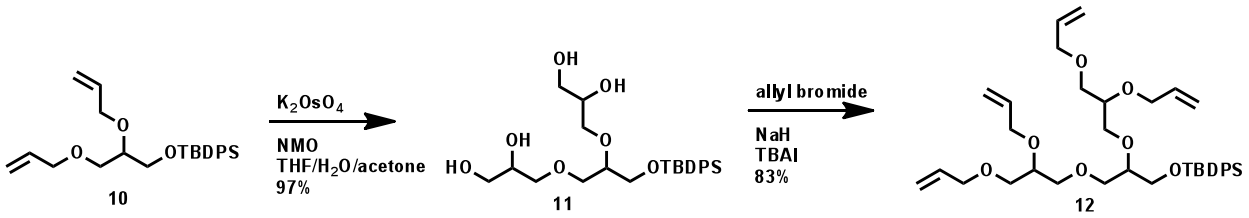
Scheme 3



7 8 9 10

Allylation of **9** with allyl bromide, sodium hydride, and tetrabutylammonium iodide (TBAI) as a catalyst afforded clear oil **10** in 93% yield.[13] This was purified by column chromatography and appeared to contain an impurity by ^{1}H NMR. It was determined to be a very small amount of **8**. It was hypothesized that the impurity was from the allylation of tert-butyldiphenylsilanol acquired from the hydrolysis of the tert-butyldiphenylsilylchloride in the initial protecting group step. The two products had similar R_f values (0.8 in 9:1 v:v petroleum ether:ethyl acetate), so they were not separated. The dihydroxylation/allylation procedure represents one generation of dendrimer growth.

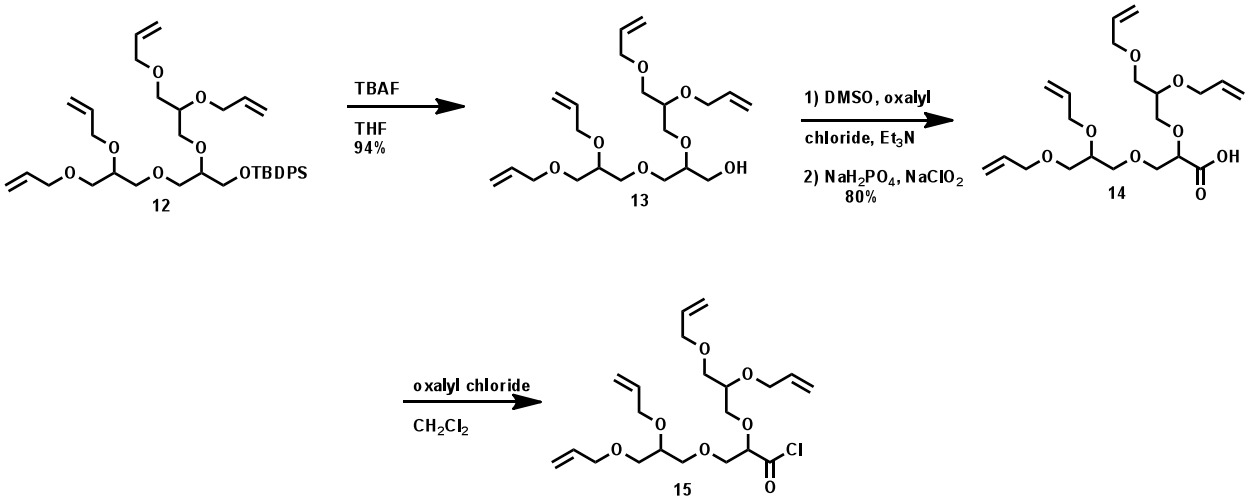
Scheme 4



Dihydroxylation of **10** using the same conditions as formation of **9** afforded **11** in 97% yield (Scheme 4). Subsequent allylation of **11** afforded a yellow oil which was separated by column chromatography to afford **12** in 83% yield which was characterized by ^{1}H NMR to be pure. The yield was much lower than the previous allylation reaction. Many optimization reactions were done to help improve the yield of **12.** It was hypothesized the lower yield resulted from the impurities from subsequent steps being removed by the chromatography because of the difference in polarity of the larger molecule. Also, **11** is fairly soluble in water, therefore during extraction with ethyl acetate some may have been left behind in the water layer. Even though careful attention was paid to all steps, it was never figured out why this step gave such a low yield. It was hypothesized that the NMO would not need to be removed to do the allylation step, because the NMO would be removed via column chromatography during the purification of the

allylated material. However, this did not help the yield either, therefore eliminating the option that some of the dihydroxylated material was left in the water layer during extraction.

Scheme 5

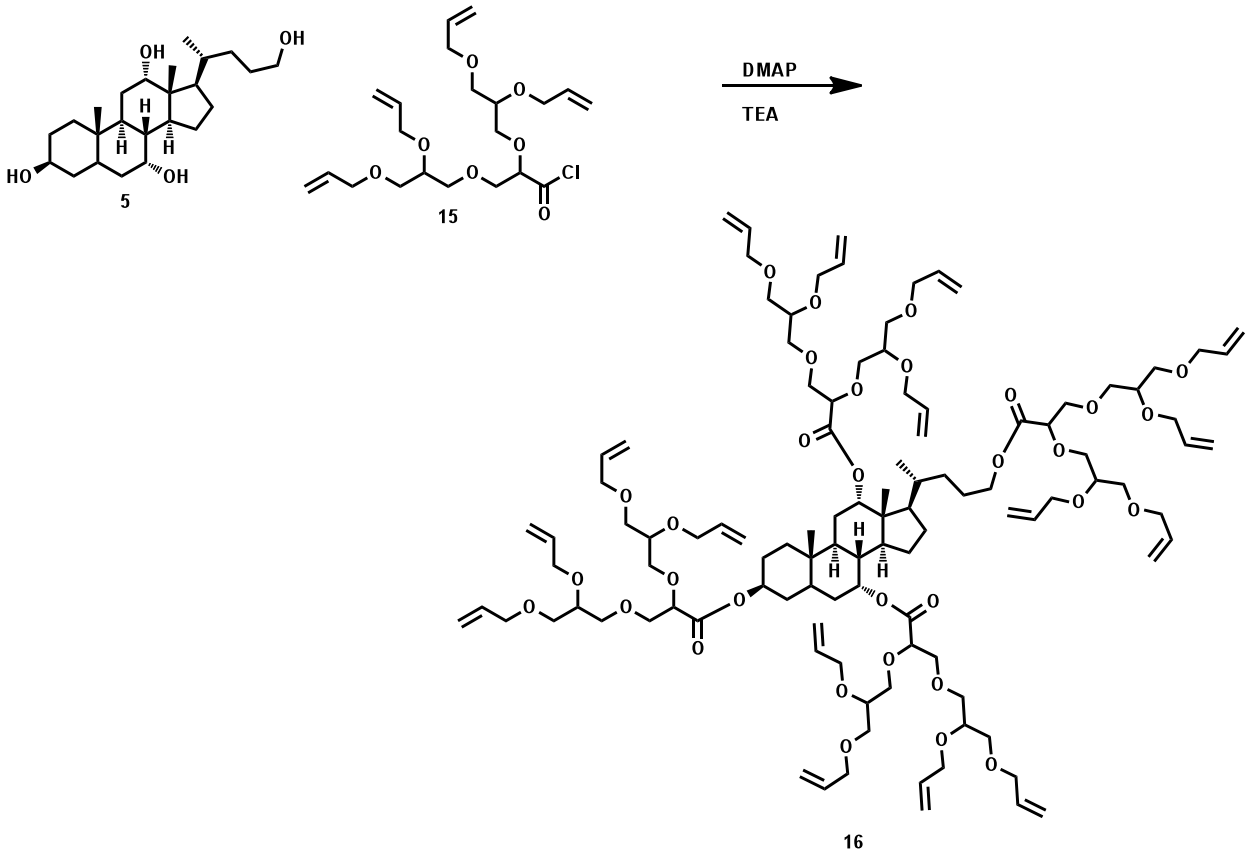


Removal of the protecting group with tetrabutylammonium fluoride afforded the alcohol **13** in 94% yield. (Scheme 5) Swern oxidation using oxalyl chloride and dimethylsulfoxide with triethylamine and subsequent oxidation with NaH_2PO_4 and $NaClO_2$ afforded the acid **14** in 80% yield. The acid was reacted with oxalyl chloride to afford the acid chloride **15**. Because of time constraints, **15** was not fully purified and was carried onto the next step to see if coupling to the steroid would occur.

Reduced cholic acid template **5** was reacted with 4.1 molar equivalents of **15** in triethylamine with a catalytic amount of 4-dimethylamino pyridine (DMAP) to form macromolecule **16** with 32 allyl groups (Scheme 6).[14] Results suggested that at least two of the four possible attachments occurred according to analysis by matrix assisted laser-desorption ionization (MALDI). ^{1}H NMR analysis also showed some attachment of the dendron to the template. Many other conditions were tried in order to get the other two attachments, but none

were found. After doing more analysis on the cholic acid core, it was determined that this would not be a suitable core for molecular imprinting and was subsequently abandoned.

Scheme 6



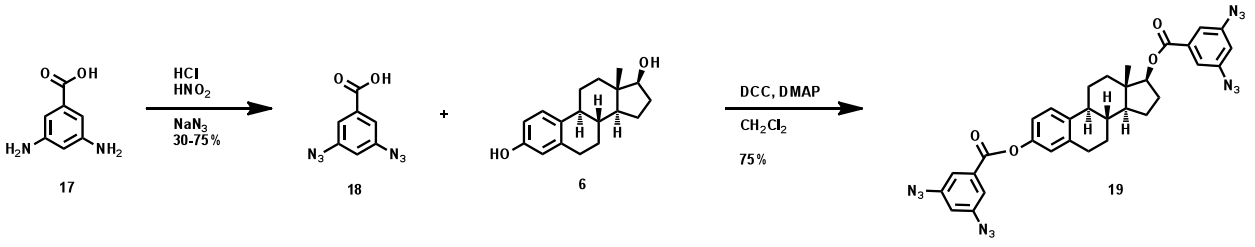
2.3 Changing of Core and Increased Dendrimer Size

The reduced cholic acid core, after being deemed not useful for this experiment, was abandoned. Estradiol was then selected as the new core for its ease of availability and unique properties. After seeing how difficult it was to attach the acid chloride to the reduced cholic acid, a new method was envisioned to help alleviate that downfall. Click chemistry is a subset of chemistry that allows for easy and fast attachment of small molecules with reactions that require little to no workup and react quickly. The Huisgen 1,3-dipolar cycloaddition of alkynes and

azides using a copper catalyst is one example of a click reaction. One simply has to introduce an alkyne with an azide together and they will for a triazole very rapidly and efficiently.

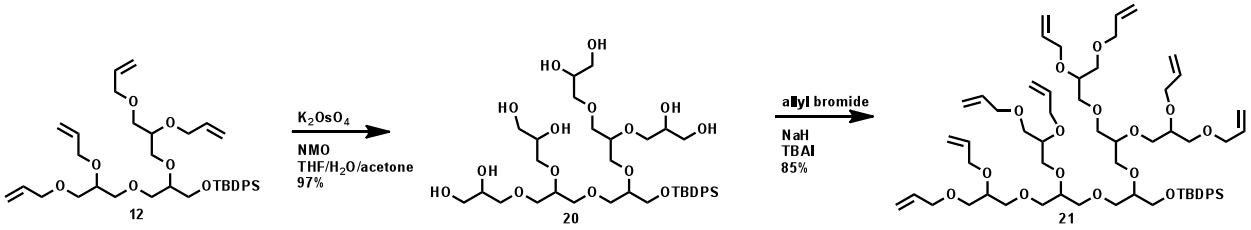
The estradiol molecule already suffered from only having two attachment points. It was envisioned that this could be overcome by attaching a molecule that has two azides on it in order to help with the number of available dendrimers. It was determined that the easiest synthetic route would have the azides on the estradiol molecule and the alkyne on the dendrons. Two azides with two attachments could allow for a maximum of four dendrons on the estradiol molecule. As outlined in scheme **7**, 3,5-diaminobenzoic acid **15** was transformed to 3,5-diazidobenzoic acid using a modified Sandmeyer reaction. **16** was formed by treating **15** with 6M HCl in a solution of 5 equivalents of sodium azide with sodium nitrite very slowly. The azide had to be added in the beginning as there is a known reaction of the decomposition of azides with sodium nitrite. **16** was formed in very sporadic yields, ranging from 30-75%. It was purified via column chromatography to afford an off-white solid that was pure via ^{1}H NMR. **16** was added to estradiol **6** in dry CH_2Cl_2 with 2.5 equivalents of DMAP. N-N'-dicyclohexylcarbodimide (DCC) was slowly added to the reaction mixture over a period of 6 hours for ester formation. Upon analysis with TLC, it was determined that the reaction had completed, with **17** being formed in 75% yield after purification with silica gel chromatography. It was noticed that the spot directly below the product was one attachment of **16**, and it could not be pushed further even if more of the reactants were added.

Scheme 7



With the core ready for attachment with the estradiol/azide component successfully synthesized, the focus was shifted to making an alkyne dendron derivative. The TBDPS protecting group of the dendrons synthesized for the reduced cholic acid were still ideal because of their easy removal and subsequent modification to other functional groups. However, the number of alkenes on each dendron was only four, and this was calculated to be far too few for efficient crosslinking in a subsequent step. Therefore, it was decided to grow the generation of the dendrimer to 16 alkenes, or two more generations. As outlined in Scheme 8, this process was very similar to the process of growing the dendrimer **12**, owing to the repetitive nature of the allylation/dihydroxylation steps.

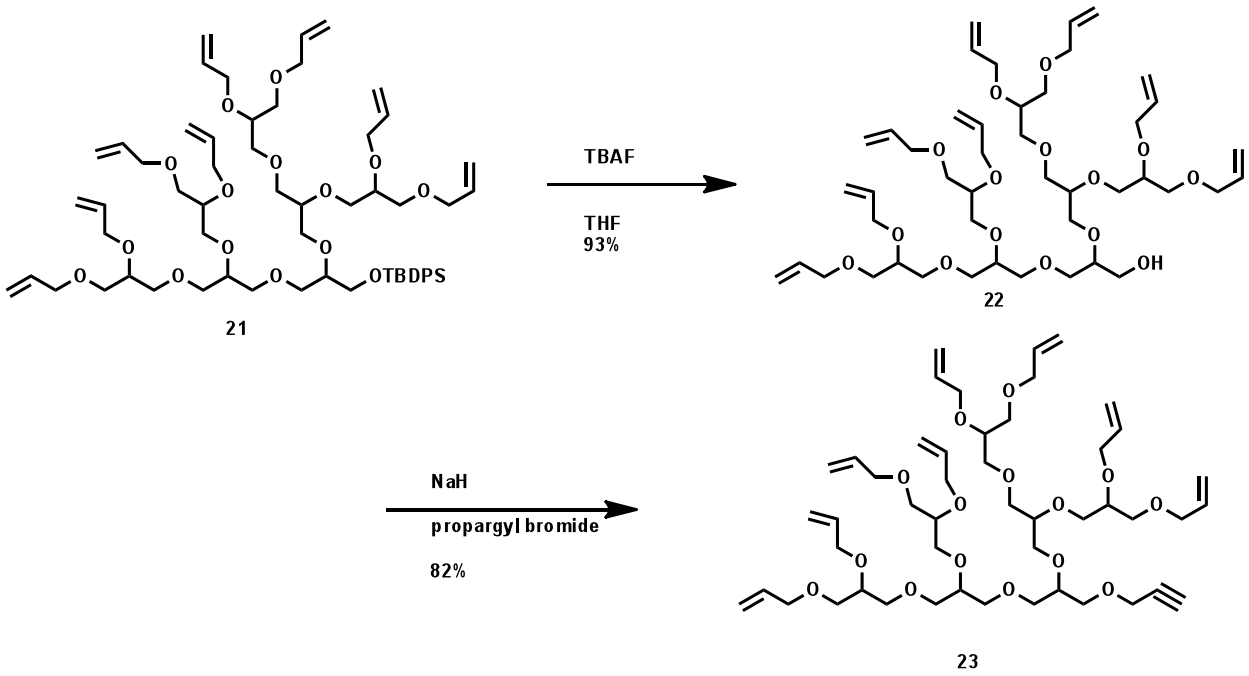
Scheme 8



Compound **12** was dihydroxylated using the standard Upjohn conditions to afford **20** in 97% yield. **20** was then allylated using the standard conditions to afford **21** in 85 percent yield. It was purified via column chromatography and integrated well via ^{1}H NMR, but MALDI analysis showed some impurities of former generation dendrimers. In order to prepare the dendron for core attachment, the protecting group had to be removed and the molecule be modified with an alkyne. This was done as outlined in Scheme 9. **21** was treated with tetrabutyl ammonium fluoride (TBAF) in THF for 8 hours. TLC analysis showed the reaction was complete after five hours, but it was run for a little longer to ensure total completion. **22** was formed in 93% yield after column purification. It was then treated with sodium hydride and

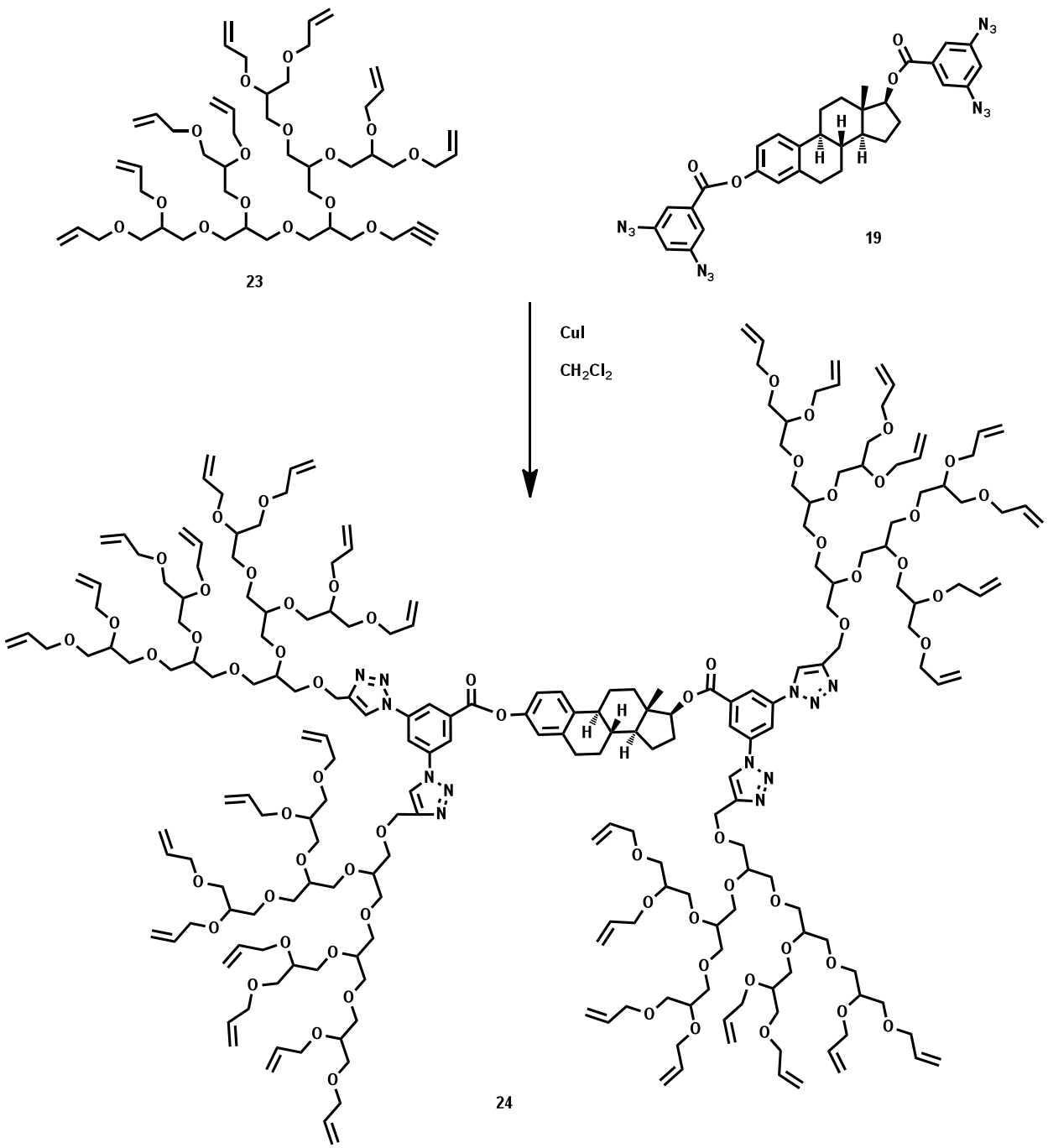
propargyl bromide to create the alkyne functionality. TBAI was not added as previous attempts of adding it only made the yields lower for unknown reasons. After optimization of the reaction, it was found that the highest yield available was 82%. The product was a yellow oil, which was not the expected result as all the other compounds were clear oils after purification.

Scheme 9



Alkyne **23** was ready for coupling to the estradiol template. A literature survey was done and it was found that the best coupling method included using copper(I) iodide in refluxing methylene chloride to afford the highest yields (Scheme 10). After reaction of two days, it was determined that the reaction should be worked up as it appeared that the reaction was completed by MALDI.

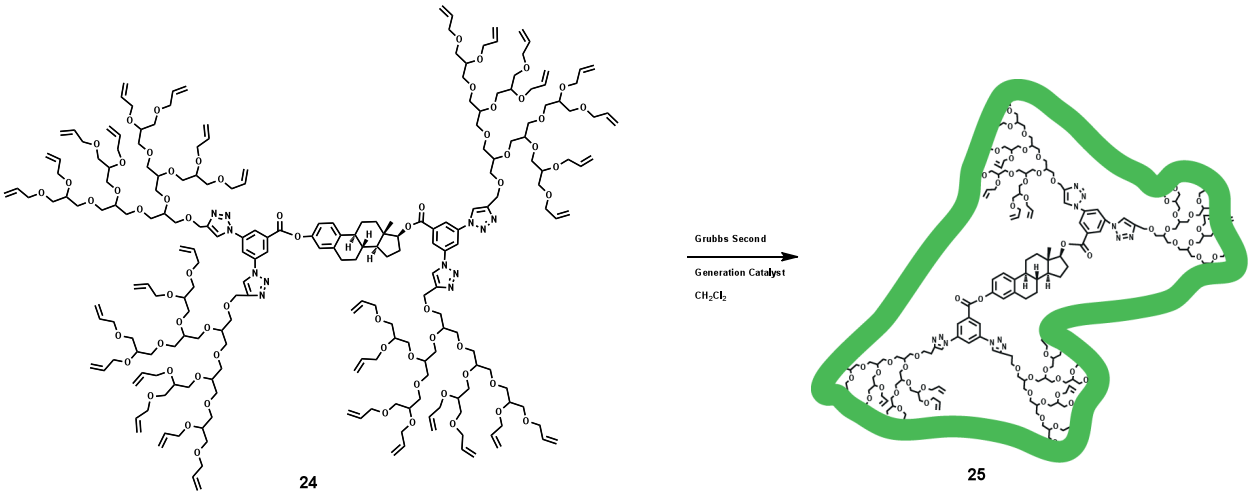
Scheme 10



After extensive purification of **24** using SEC chromatography, it was determined that the reaction should be moved forward to crosslinking. **24** was crosslinked using Grubbs' second generation catalyst at a concentration of 1E-5 M. The reason the concentration had to be so

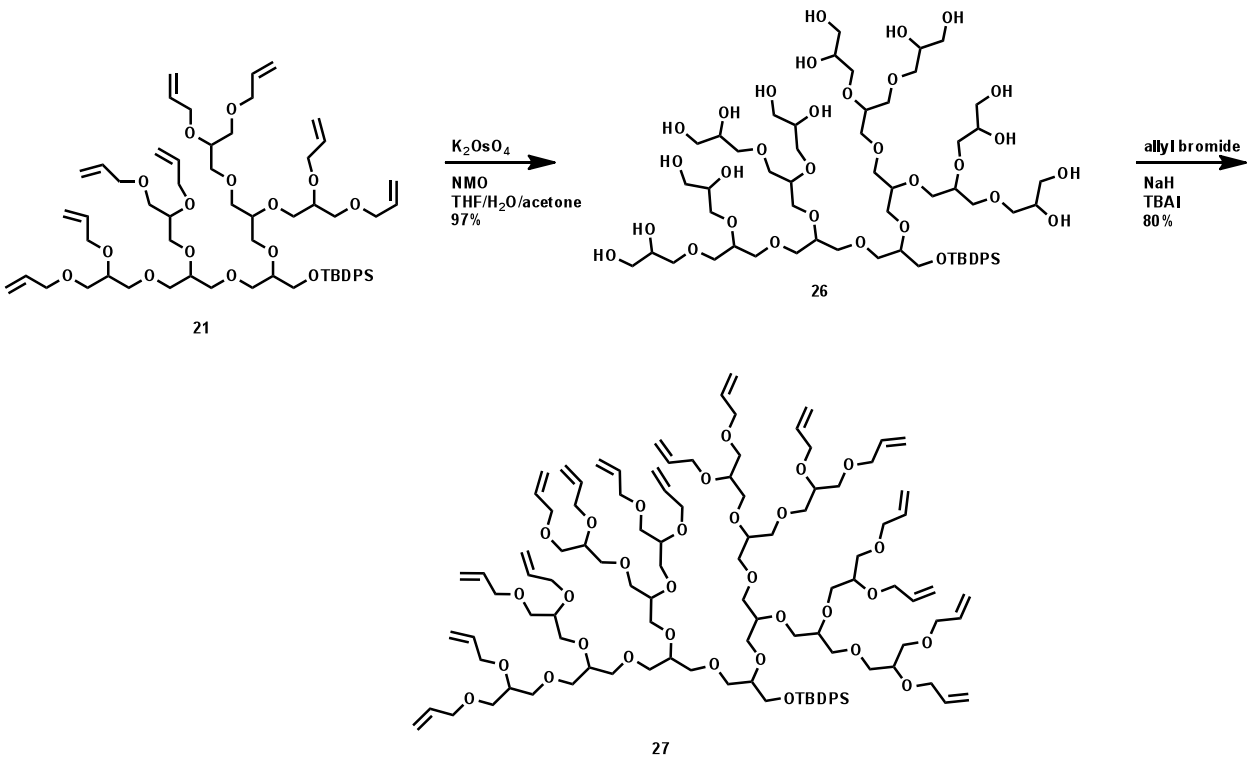
dilute was to ensure that there was no intermolecular crosslinking, only intramolecular crosslinking (Scheme 11). This was allowed to crosslink for approximately 5 days, and was carefully monitored using MALDI to see when the reaction had stopped crosslinking. After crosslinking had completed and the material was purified, it was shown that after coring the crosslinked material using KOH/EtOH, the dendrimer fell completely apart and there was not much crosslinking in between the dendrons. This was hypothesized to be from the dendrons not being large enough to reach across the molecule to crosslink with each other.

Scheme 11



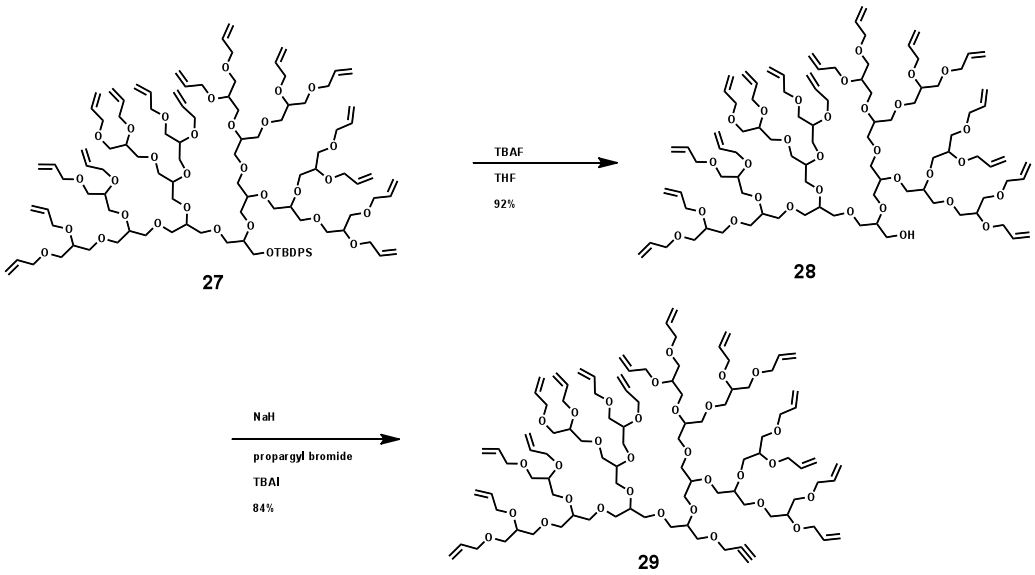
It was then envisioned that to correct this too small of a dendron, one must have a larger generation dendron. The synthesis of the larger generation dendrimer is outlined in Scheme 12. The process of allylation/dihydroxylation was repeated as before. However, because the molecule was very large, it contained more hydrophilic groups, and was therefore much harder to extract into the ethyl acetate layer. A rigorous extraction with 10% methanol / chloroform was used to remove the polyol with ease. The subsequent allylation step was done with fairly good yields considering the number of allylations (16) that had to be performed. **27** was formed in around 80% yield with fairly simple purification via column chromatography.

Scheme 12



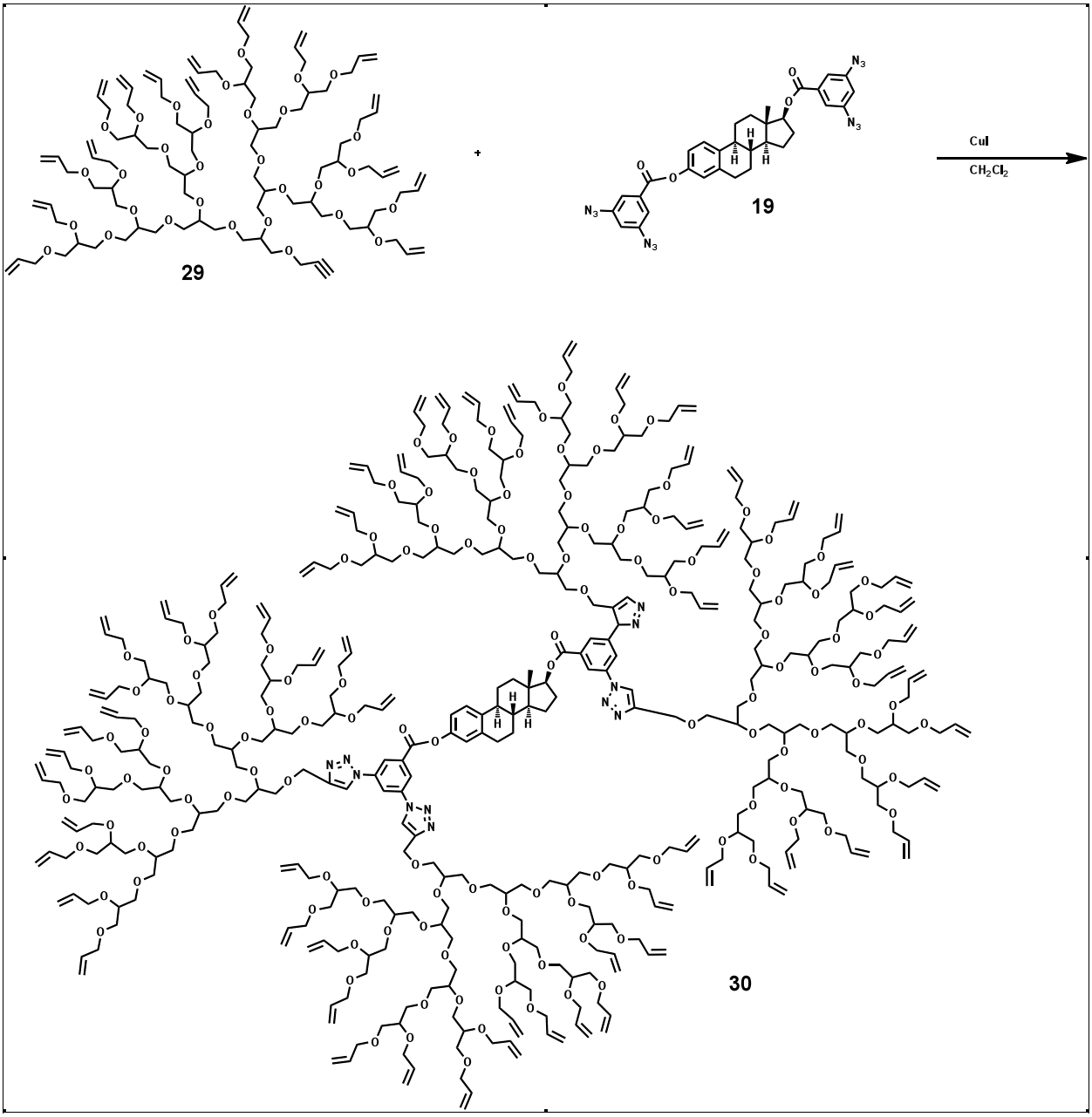
27 was then deprotected using TBAF in THF in 92% yield and subsequently proparglyated using propargyl bromide with sodium hydride as base to form **29** in 84% yield.

Scheme 13



Alkyne **29** and estradiol core **19** were coupled using the same conditions as the previous generation dendron. This afforded **30** as a dendrimer with 64 alkenes available for crosslinking (Scheme 14).

Scheme 14



After close monitoring of the reaction using MALDI (Figure 3) it could be seen that the majority of the cycloadditions had occurred.

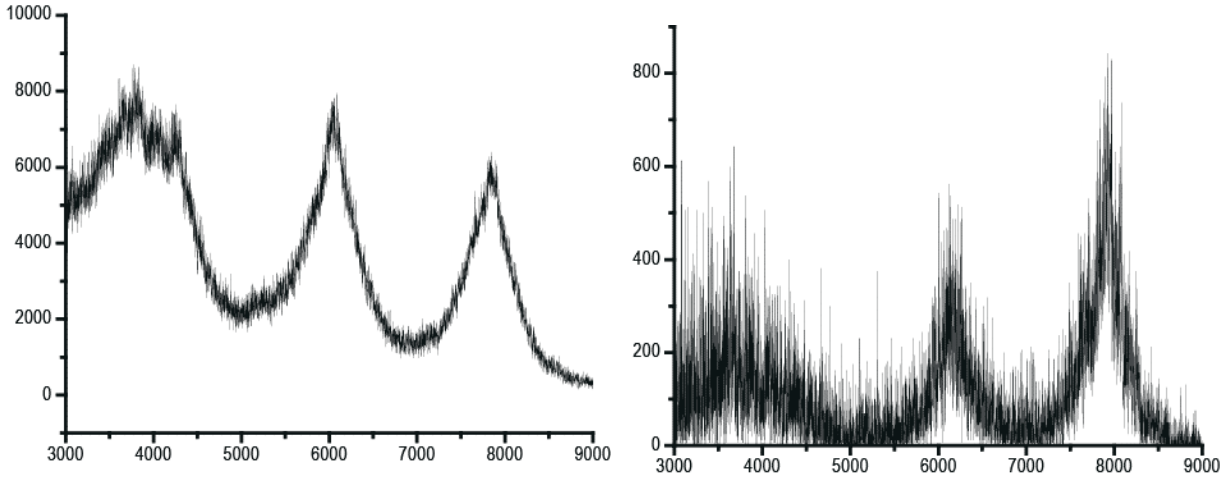


Figure 3. Left- Cycloaddition reaction after four hours. Right – Cycloaddition after 24 hours

After purification of **30** using an SEC column, it was subjected to the same crosslinking conditions as **24**. **30** was diluted to a concentration of 1E-5 M in methylene chloride and was allowed to reflux for 5 days with 10 mol % Grubbs' second generation catalyst, when it appeared that the crosslinking had completed (Figure 4).

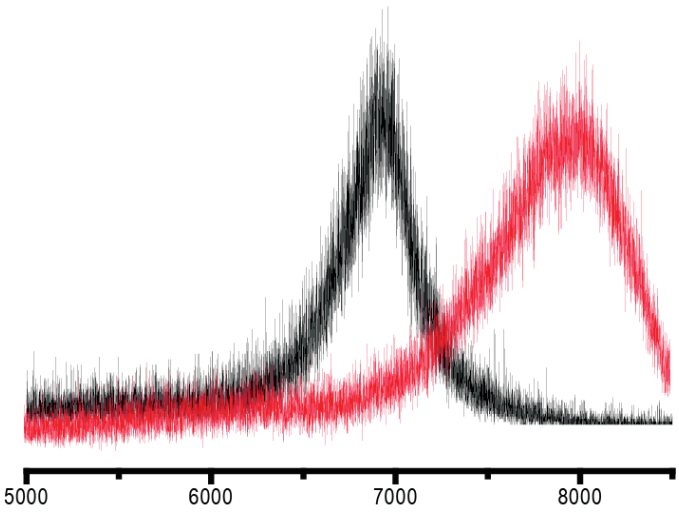


Figure 4. Uncrosslinked (red) and crosslinked (black) MALDI of **30**

The crosslinked dendrimer was then cored using the same KOH/EtOH solution as before. This time, it was more promising because there was more intramolecular crosslinking, however it could still be seen that some of the dendrons were not being held together.

2.4 Conclusions and Future Directions

Because of the unsuccessful nature of this approach, it is concluded that an alternative method be used for the dendrimer. This can be done by using a hyperbranched polymer or some sort of polymer that isn't so reactive. One of the most difficult aspects of this project was the instability of the polyalkenes. If not monitored carefully, they would crosslink in the flasks in approximately a week. Therefore, it was very difficult to keep material available to perform the reactions, and it was necessary to remake lots of material.

CHAPTER 3:
HYPERBRANCHED POLYMERS

3.1 Introduction

Hyperbranched polymers[15] offer a different approach to dendrimer synthesis. Not only are they very similar in structure to dendrimers, but they also are synthesized in one step, unlike the multi-step synthesis needed for dendrimers. This allows for a very efficient synthesis of the dendrons. However, there are some drawbacks to using this method. For instance, the hyperbranched polymer would not be monodisperse like the stepwise dendrimer would be. Also, if one were able to attach the hyperbranched dendron to a template, the resultant MIP would be polydisperse.

There are many different types of hyperbranched polymers,[16] but since our group was working on dendrimers based on polyethers, research was focused on the known hyperbranched polymerization of glycidol with an alkoxide monomer. A general scheme[17] is shown in Figure 5.

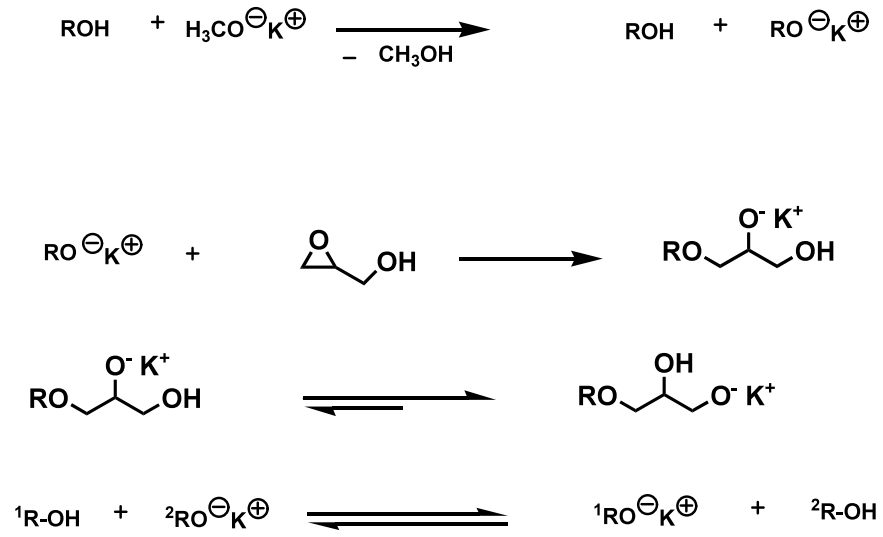


Figure 5. Hyperbranched polymerization using glycidol as a monomer.

The mechanism of polymerization is as follows: Deprotonation of an alcohol with a base leads to a generated alkoxide anion. This newly formed alkoxide anion nucleophilically attacks

the less hindered side of the glycidol, opening the epoxide ring to generate a new alkoxide. Cation transfer may occur from the secondary alkoxide to the primary alkoxide. The newly formed alkoxide attacks another molecule of glycidol, and the process repeats itself until glycidol runs out leading to a highly branched polyether structure. Termination occurs from the transfer of the alkoxide to another molecule of initiator, or acidification during workup. A representative structure of a hyperbranched polymer is shown in Figure 6.

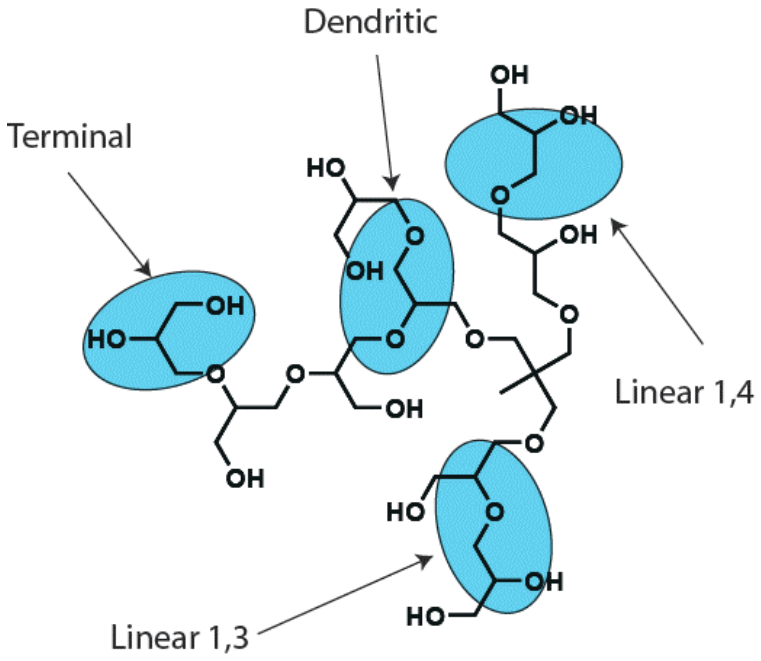


Figure 6. Different branching structures of a hyperbranched polymer.

It can be seen in Figure 4 there are three main types of branching structures. The linear 1,4 addition is most prevalent because the transfer from the secondary alkoxide to the primary alkoxide is favored. A dendritic structure will be formed when addition occurs from both ends, resulting from alkoxide transfer from another molecule. The linear 1,3 addition is the least common because of the alkoxide transfer.[17]

Unfortunately, there is a side reaction of the polymerization that is unwanted. If the reaction is allowed to equilibrate and glycidol is added too quickly, unwanted cyclic structures

are formed. This occurs because alkoxide transfer occurs from the wanted polymer to the hydroxyl proton of the glycidol (Figure 7). The glycidol is then a reactive intermediate, and will polymerize with more available glycidol, until nucleophilically attacking the initial epoxide monomer to form a cyclic structure.

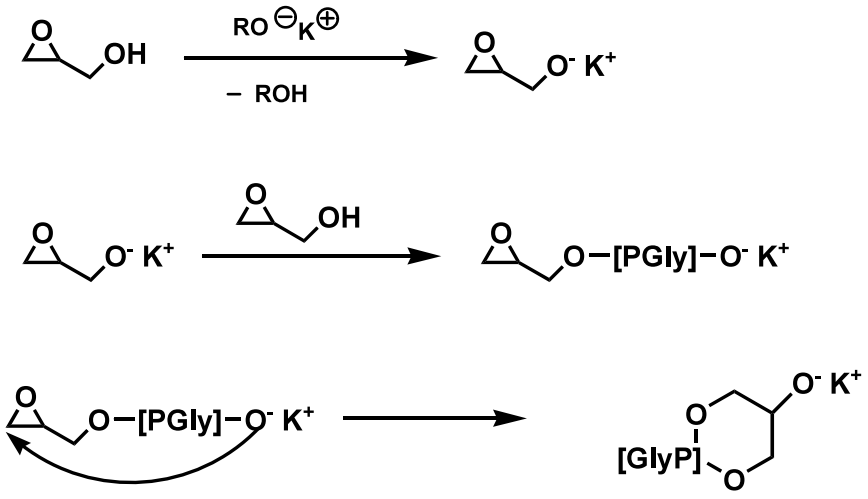


Figure 7. Formation of cyclic structures from fast addition of glycidol.

3.2 Optimizing Conditions for Hyperbranched Polymerization

The synthesis of a hyperbranched polymer appeared to be fairly simple, as Frey[17] and coworkers were able to generate hyperbranched polymers with low polydispersities using an appropriate monomer:initiatior ratio. In their procedure, potassium methylate was used to deprotonate the initiator, trimethylolpropane. Frey also stated that the best results were from only partially deprotonating the initiator 10%. The polymerization was done neat at 95°C with only glycidol and initiator present. Experimental details were rather vague, so it was important to optimize the conditions for polymerization. Although these reactions can be done in one step, the addition is quite slow and may take over two days to do the entire polymerization process.

The first attempt of polymerization of trimethylol propane using potassium methoxide as base resulted in mild success. According to MALDI (Figure 8), polymerization had indeed occurred, but there was unwanted cyclic material.

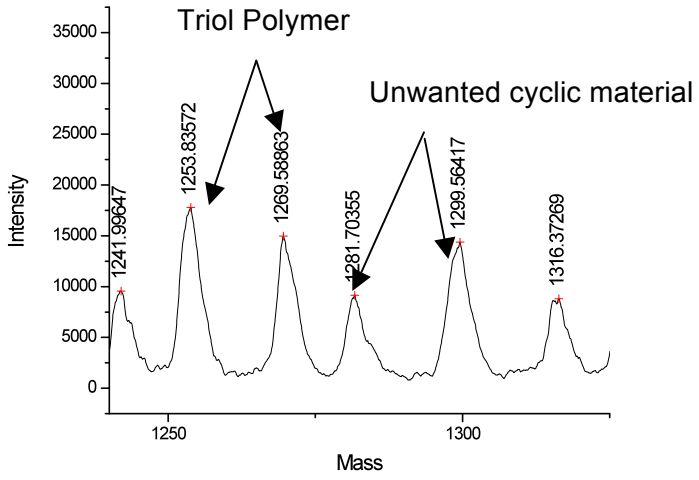


Figure 8. Initial attempts produced a large amount of unwanted material

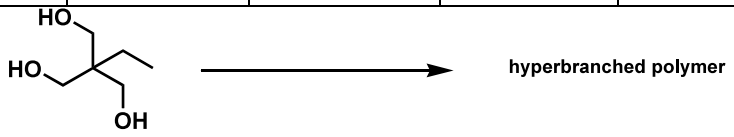
The selection of base was found to be quite problematic. The procedure called for partially deprotonating the initiator (10%) using potassium methoxide, and subsequently heating the reaction mixture to evaporate resultant methanol. A better selection for base would be one that creates a byproduct that could be removed fairly quickly. This base was sodium hydride, as its byproduct is hydrogen gas. It showed improved results.

Other steps were taken to optimize the polymerization conditions. It was noticed that the addition of glycidol may have been occurring too quickly. Therefore, it was added very slowly via syringe pump. Using this method, it was noticed that there was less unwanted cyclic material.

Stirring efficiency was also a problem. After a while, the reaction mixture would become very thick and the stir bar would not be able to mix the neat solution. Therefore, diglyme was added as an emulsifier because of its high boiling point necessary for the reaction conditions. Starting alkoxide was fairly soluble in the diglyme, but as the polymerization occurred, the polymer would crash out of the emulsion. Diglyme helped, but the solution still became rather viscous. In order to help stirring efficiency, a mechanical stirrer was attached to a 3 necked round bottom flask. This greatly improved the ratio of polymer to cyclic byproduct. A summary of the hyperbranched polymerization of trimethylolpropane is in Scheme 15.

Scheme 15

Addition Rate (mL/hr)	Solvent	Stirring	Base Used	Polymer:Cyclic Ratio
0.5	neat	stir bar	KOMe	2:1
1.0	neat	stir bar	KOMe	1:1
0.5	diglyme	stir bar	KOMe	2:1
1.0	diglyme	stir bar	KOMe	1:1
1.0	neat	stir bar	NaH	5:1
1.0	diglyme	stir bar	NaH	10:1
1.0	diglyme	mechanical	NaH	25:1
0.5	diglyme	mechanical	NaH	50:1



These optimized conditions were employed for further alcohols. Pentaerithritol, a tetra-ol, and many other alcohols were tried to see if this procedure can be generalized. Results of the polymerization of pentaerithritol are shown in Figure 9.

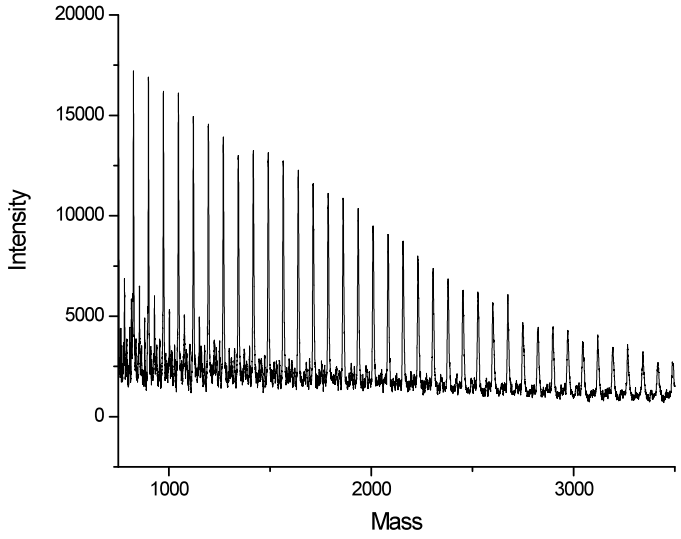


Figure 9. Hyperbranched polymerization of pentaerithritol.

In order to calculate the polydispersity of the polymerization, allylation of the hyperbranched material was done because the gel permeation chromatography (GPC) instrument was not equipped with a water column. The procedure involved using allyl chloride and a catalytic amount of tetrabutylammonium bromide in a 50% solution of sodium hydroxide. After workup, the viscosity of the allylated polymer decreased noticeably. Dialysis was performed using benzoylated cellulose to remove small polymers and unreacted starting material. GPC measurements were made and the resultant polymer was found to have a polydispersity index of 1.38. (Figure 10 on next page) Although this number is relatively good, it is not a good representation of the original polymer because it was extracted after the allylation procedure.

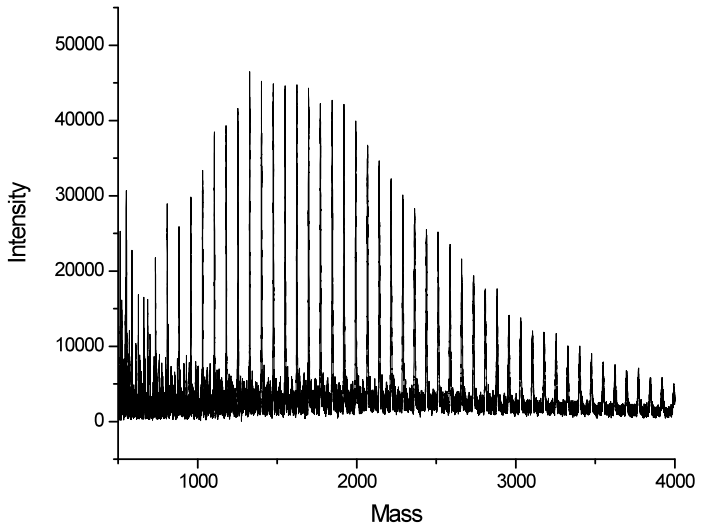


Figure 10 Hyperbranched polymer after allylation and dialysis.

3.3 Conclusion and Future Work

Much work and effort was accomplished in trying to optimize conditions necessary for the hyperbranched polymerization of glycidol using an alkoxide initiator. It was found that the optimal conditions included using sodium hydride as a base, emulsifying the solution with diglyme, and stirring rapidly with a mechanical stirrer. Under these conditions, polymerization of any alkoxide species will result in relatively high yields and low polydispersities.

In order to use hyperbranched polymers as dendrons for molecular imprinting, they must contain a synthetic handle to attach to the template. This handle is much more difficult to obtain, because of the necessary polymerization conditions (basic, high temperatures) will be detrimental to formation of an acid analogous to Scheme 5. Therefore, a starting polyol must be contain a group that is stable to highly basic conditions. It may be possible to start the polymerization using propargyl alcohol as an initiator, but it is difficult to predict the stability of these molecules under strongly basic conditions. Such options will be explored in the future.

CHAPTER 4:
EXPERIMENTAL

4.1 General Methods

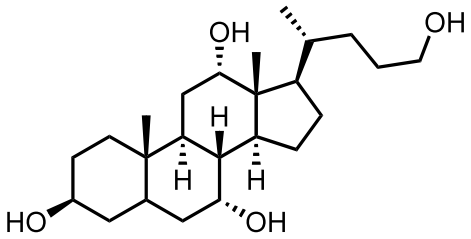
All reactions were performed under a dry nitrogen atmosphere unless otherwise noted. Temperatures reported are uncorrected. Most solvents and reagents used were purchased from Aldrich and used out of the bottle without purification. Tetrahydrofuran (THF) was distilled over sodium and benzophenone. Chloroform, methylene chloride and dimethyl formamide were stored over 4 Å molecular sieves.

Thin layer chromatography was performed on 0.2 mm silica gel 60 F_{254} plastic sheets (Merck). Column chromatography was performed on Merck 40-60 μm silica following the procedure of Still[18], and all solvent ratios are v:v. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a 500 or 400 MHz Varian Unity Spectrometer at the Varian Oxford Instruments Center for Excellence (VOICE) NMR laboratory at the University of Illinois Urbana-Champaign. ^{1}H NMR coupling constants are reported in hertz (Hz). All ^{1}H NMR spectra were referenced to residual solvent peak at 7.26 for chloroform-*d*, 2.50 for dimethylsulfoxide-d_6 , or 3.31 ppm for methanol-d_4. Carbon nuclear resonance (^{13}C NMR) was obtained on the same instruments at 125.7 and 100 MHz respectively and was referenced to 77.0 ppm in chloroform-*d,* 39.51 ppm for dimethylsulfoxide-d_6 , and 49.15 ppm for methanol-d_4. Unless stated, the ^{1}H and ^{13}C NMR spectra were acquired in $CDCl_3$.

All mass spectra were obtained at the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois, Urbana-Champaign. Matrix Assisted Laser Desorption Ionization (MALDI) spectra were obtained using an Applied Biosystems Voyager-DE STR spectrometer using either 2-(4-Hydroxyphenylazo)benzoic acid or α-cyano-4-

hydroxycinnamic acid as a matrix. Analytical gel-permeation chromatography (GPC) was perfomed on a Waters Sytragel HR3 triple column coupled with a Viscotek TDA model 300 triple detector array. Molecular weights (M_w and M_n) and polydispersity values were based on using polystyrene standards as calibrates.

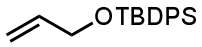
4.2 Experimental Procedures



5

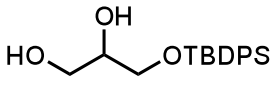
17-(4-Hydroxy-1-methyl-butyl)-10,13-dimethyl-hexadecahydro-cyclopenta[a]phenanthrene-3,7,12-triol (5).[10] Cholic acid (1.5 g, 3.67 mmol) was dissolved in 40 mL of dry THF in a 100 mL round bottom flask. Triethylamine (2.0 mL, 15 mmol) was added followed by ethylchloroformate (1.2 mL, 125 mmol) dropwise. The reaction was allowed to stir at room temperature for three hours. During this process, the reaction turned a yellow color. A solution containing 1.6 g (42.29 mmol) of $NaBH_4$ in 16 mL distilled H_2O was added dropwise. Upon addition, the reaction bubbled. The reaction was stirred overnight. 300 mL of water was added in the morning and the solution was neutralized with 1.0M HCl, and extracted with (3 x 100 mL) ethyl acetate, washed with (1 x 100 mL) distilled water, dried with sodium

sulfate and rotary evaporated to afford a white solid in 60% yield. MP and ^{1}H NMR similar to reference.



8

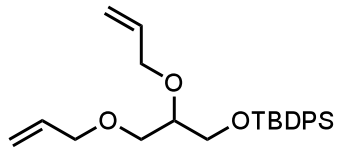
Allyloxy-tert-butyl-diphenyl-silane (8).[12] To a flame dried 65 mL round bottom flask purged with nitrogen was added 4.01 g (43.76 mmol) of tert-butyldiphenylsilyl chloride. Imidazole (4.0 g, 58.35 mmol) was added and the reaction was dissolved in 25 mL of dry dimethylformamide. The reaction was stirred until everything was in solution. Allyl alcohol (2.5 g, 43.76 mmol) was added slowly and the reaction was allowed to run overnight. Reaction was shown to be completed by TLC, and was subsequently extracted with (3 x 50 mL) ethyl ether, and washed with (2 x 100 mL) of distilled water. The organic layer was dried with sodium sulfate, and reduced to afford 4.2 g (97 % yield) of a clear liquid. ^{1}H NMR (500 MHz) δ: 7.69 (m, 4H), 7.40 (m, 6H), 5.93 (ddt, 1H, *J* = 17.1, 10.4, 4.2) 5.38 (ddt, 1H, *J* = 17.1, 2, -1.5), 5.38 (ddt, 1H, *J* = 11, 2, -1.3), 4.21 (m, 2H), 1.07 (s, 9H). ^{13}C: 137.2, 135.7, 133.9, 129.9, 127.9, 114.1, 64.8, 27.0, 19.4.



9

3-(tert-Butyl-diphenyl-silanyloxy)-propane-1,2-diol (9) To a flame dried 100 mL round bottomed flask purged with nitrogen was added 4.00 g (13.49 mmol) of **8**. 15 mL of

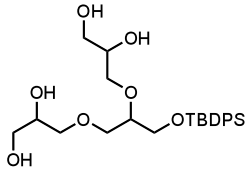
distilled water, 15 mL of THF, and 15 mL of acetone were added to the flask. A magnetic stir bar was added and the NMO (1.73 g, 14.84 mmol) was added. The reaction mixture was cooled to 0°C and potassium osmate dihydrate (5 mg) was added to the reaction mixture. WARNING: OSMIUM PRODUCTS ARE VERY HAZARDOUS! Reaction allowed to reach room temperature and reacted for 20 hours. After 20 hours, reaction was complete by TLC analysis. Solvent evaporated under reduced pressure and extracted (3x100 mL) of ethyl acetate. Washed (1 x 100mL) with distilled water, organic layer dried over Na_2SO_4, and evaporated to afford 4.325 g (95%) of a tan solid. ^{1}H NMR (DMSO- d_6 500 MHz) δ: 7.42 (m, 4H), 7.36 (m, 6H), 4.66 (m, 1H), 4.48 (m, 1H), 3.59 (m, 2H), 3.54 (m, 1H) 3.42 (m, 2H). ^{13}C: 135.8, 134.0, 130.5, 128.5, 72.7, 66.0, 63.5, 27.3, 19.5.



10

(2,3-Bis-allyloxy-propoxy)-tert-butyl-diphenyl-silane (10)[13] To a flame dried 100 mL round bottom flask purged with nitrogen was added alcohol **9** (2.5 g, 7.56 mmol), allyl bromide (11 mL, 121 mmol), and tetrabutylammonium iodide (50 mg, 0.135 mmol). 50 mL of dry THF from the still was added and the reaction was cooled to 0°C with an ice water bath. NaH (1.7 g, 45 mmol) was added very slowly while stirring. The reaction mixture went from a clear color to a milky color. Upon completion of addition, the reaction was allowed to warm to room temperature and react for 20 hours. After 20 hours, reaction was complete by TLC. The reaction was once again cooled to 0°C and 50 mL of H_2O was added to quench excess sodium hydride. Extracted with ethyl acetate (3x 75 mL) and washed with distilled water (1x 100 mL). Dried organic layer with Na_2SO_4 and evaporated to afford a yellow oil. Ran a silica gel column eluting

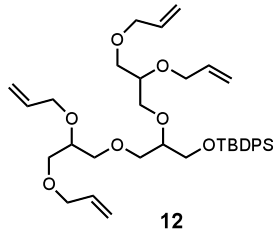
with 100% petroleum ether (PE). Loaded compound onto column, and eluted using 0.5 L of PE followed by 1.0 L of 9:1 PE:EtOAc. Obtained 3.0371g (97% yield) that appeared to contain a small impurity by ^{1}H NMR. ^{1}H NMR (500 MHz) δ: 7.67 (m, 4H), 7.38 (m, 6H), 5.89 (m, 2H), 5.25 (m, 2H), 5.15 (m, 2H), 4.09 (m, 2H), 4.01 (m, 2H), 3.70 (m, 2H), 3.63 (m, 2H), 3.54 (m, 1H), 1.05 (s, 9H). ^{13}C: 136.2, 135.9, 135.5, 135.1, 133.7, 127.9, 78.8, 72.6, 70.4, 63.6, 27.0, 19.4.



11

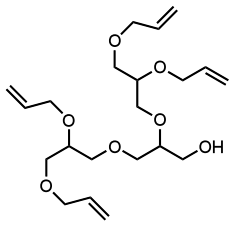
3-[3-(tert-Butyl-diphenyl-silanyloxy)-2-(2,3-dihydroxy-propoxy)-propoxy]-propane-1,2-diol (11) To a dry 200 mL round bottom flask purged with nitrogen was added 2.5 g (6.08 mmol) of **10**. 25 mL of acetone, 25 mL of THF, and 25 mL of distilled water were added along with a magnetic stir bar to the reaction flask. 1.72 g (15.2 mmol) of NMO was added to the reaction mixture and allowed to stir for 10 minutes. Reaction cooled to 0°C via ice water bath and 5 mg of K_2OsO_4 was added. Reaction allowed to reach room temperature and allowed to stir overnight. TLC analysis showed reaction was complete. Reaction was evaporated under reduced pressure to remove all solvents. 20 mL of distilled water was added, and extracted (3 x 50 mL) with ethyl acetate. Washed organic layer with distilled water (1 x 100 mL) and dried organic layer with Na_2SO_4 and evaporated under reduced pressure to afford 2.76 g (95% yield) of yellow oil. ^{1}H NMR (DMSO- d_6 500 MHz) δ:7.63 (m, 4H), 7.43 (m, 6H), 4.61 (m, 1H), 4.55

(m, 1H), 4.47 (m, 1H), 4.43 (m, 1H), 3.65 (m, 2H), 3.53 (m, 4H), 3.47 (m, 2H), 3.39 (m, 2H) 3.38 (m, 1H), 3.29 (m, 4H), 0.97 (s, 9H).



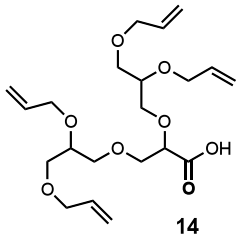
12

[2,3-Bis-(2,3-bis-allyloxy-propoxy)-propoxy]-tert-butyl-diphenyl-silane (12) To a clean dry 100 mL round bottom flask purged with nitrogen was added **11** (2.5 g, 5.22 mmol) and 25 mL of dry THF. Allyl bromide (15 mL, 167 mmol) was added with TBAI (30 mg). Reaction flask was cooled to 0°C and NaH was slowly added. Reaction allowed to reach room temperature and allowed to react overnight. Reaction showed to be complete by TLC after 20 hours. Cooled reaction to 0°C and slowly quenched with 15 mL of distilled water. Extracted with ethyl acetate (3 x 100 mL) and washed with water (1 x 100 mL), dried organic layer with sodium sulfate, and evaporated to produce a yellow oil. Ran silica gel column eluting with 100 % petroleum ether. Loaded compound onto column and eluted with 0.5 L petroleum ether, followed by 1.5 L 9:1 PE:EtOAc to afford 2.42 g (73 % yield) of a slightly yellow oil that was determined to be pure by ^{1}H NMR. ^{1}H NMR (500 MHz) δ: 7.67 (m, 4H), 7.40 (m, 6H), 5.88 (m, 4H), 5.25 (m, 4H), 5.14 (m, 4H), 4.11 (m, 4H), 3.98 (m, 4H), 3.71 (m, 2H), 3.37-3.70 (m, 13H), 1.05 (s, 9H). ^{13}C: 135.8, 135.5, 135.4, 135.1, 135.0, 129.9, 80.4, 72.5, 71.9, 71.6. 71.5, 63.9, 26.9, 19.3.



13

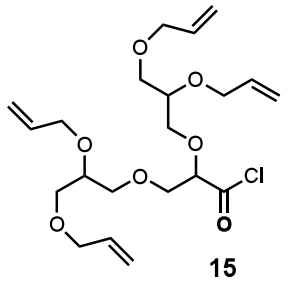
2,3-Bis-(2,3-bis-allyloxy-propoxy)-propan-1-ol (13) To a flame dried 25 mL round bottom flask purged with nitrogen was added **12** (1.00 g, 1.57 mmol) and 6 mL dry THF. Tetrabutylammonium fluoride (6.0 mL, 1.0 M solution in THF, 6 mmol) was added slowly over ten minutes. Reaction stirred for 15 hours overnight. TLC analysis showed completion of reaction. 10 mL of distilled water was added and extraction with ethyl acetate (3 x 50 mL) occurred. This was washed with distilled water (1 x 100 mL) and organic layers were dried using Na_2SO_4 and were evaporated under reduced pressure. A silica gel column was run pre-eluting with 4:1 PE:EtOAc. Compound was loaded onto column and run eluting with 1.5 L 4:1 PE:EtOAc to afford 590 mg (94 %) as a clear liquid. ^{1}H NMR (500 MHz) δ: 5.88 (m, 4H), 5.25 (m, 4H), 5.14 (m, 4H), 4.11 (m, 4H), 3.98 (m, 4H), 3.71 (m, 2H), 3.37-3.70 (m, 13H), 2.90 (bs, 1H).



14

2,3-Bis-(2,3-bis-allyloxy-propoxy)-propionic acid (14) To a dry 3 necked 25 mL round bottom flask purged with nitrogen was added a septum and a thermometer. Oxalyl chloride (0.38 mL, 4.0 mmol) in 5 mL dry CH_2Cl_2 was added and the reaction was cooled to -78°C with a dry ice/acetone bath over 10 minutes. Dimethylsulfoxide (0.262 mL, 4.00 mmol) in 1 mL dry CH_2Cl_2 was added very slowly over five minutes. Stirred for ten minutes, and slowly added alcohol **13** (400 mg, 1.00 mmol) in 5 mL of CH_2Cl_2. Reaction allowed to stir for two hours.

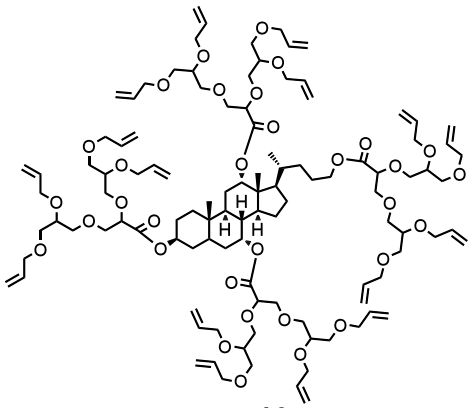
Triethylamine (0.834 mL, 6.00 mmol) was added over five minutes and reaction was allowed to warm to room temperature. Added 20 mL of water, and evaporated off solvents. Extracted with CH_2Cl_2 (3 x 50 mL) and washed with water (1 x 100 mL). Dried over sodium sulfate, and evaporated to afford 400 mg of a liquid that appeared to be the aldehyde with ^{1}H NMR. The material was then transferred to a 50 mL round bottom flask and 15 mL of tert-butanol, 5 mL of water, and 5 mL of 2-methyl-2-butene were added. The reaction was cooled to 0°C using an ice water bath. Sodium phosphate monobasic (851 mg, 6.0 mmol) and sodium chlorite (362 mg, 4.0 mmol) were added, and reaction was allowed to warm to room temperature and react overnight. Reaction was shown to be complete by TLC and 25 mL water was added and 1 M HCl was added to decrease the pH of the solution. Extracted (3 x 50 mL) with ethyl acetate, washed with water (1 x 100 mL), dried over Na_2SO_4, and evaporated. A column was run using 1:1 PE:EtOAc (1.5 L) to afford 332 mg of a liquid that appeared to be pure by ^{1}H NMR. ^{1}H NMR (500 MHz) δ: 5.89 (m, 4H), 5.27 (m, 4H), 5.15 (m, 4H), 4.12 (m, 4H), 3.99 (m, 4H), 3.73 (m, 2H), 3.37-3.70 (m, 13H).



15

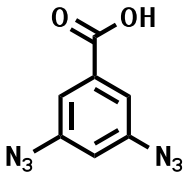
2,3-Bis-(2,3-bis-allyloxy-propoxy)-propionyl chloride (15) To a clean flame dried 25 mL round bottom flask was added **14** (300 mg, 0.72 mmol) in 10 mL CH_2Cl_2. Oxalyl chloride (0.15 mL, 1.44 mmol) was added slowly and reaction was allowed to react overnight. Reaction rotary evaporated and 25 mL of $CHCl_3$ was added 3 times and rotary evaporated. Ran through

quick silica gel plug to collect around 200 mg of product, which was used immediately in the next coupling step (was not characterized by NMR)



16

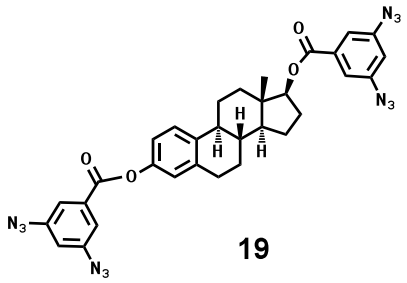
Dendron attached steroid synthesis (16)[14] To a clean dry 25 mL round bottom flask was added **5** (11 mg, 0.028 mmol) and **15** (50 mg, 0.115 mmol). 10 mL of dry THF was added as well as 4-dimethylaminopyridine (14 mg, 0.115 mol) and triethylamine (11 mg, 0.115 mmol). Reaction allowed to run overnight. Analysis by TLC showed four spots, and reaction was worked up by adding 10 mL of 1M HCl, and extracted with ethyl acetate (3 x 10 mL). Organic layer dried with sodium sulfate and rotary evaporated to afford an oil that appears to contain dendron attached steroid by ^{1}H NMR and MALDI analysis.



18

3,5-Diazidobenzoic acid (18) To a clean 500 mL RBF was added 100 mL of 6M HCl and a mechanical stirbar. This was cooled to zero degrees Celsius using an ice water bath. 3,5-

diaminobenzoic acid **17** (6.7 g, 44.0 mmol) was added and it slowly dissolved. NaN_3 (11.45g, 176 mmol) was added to the solution. Sodium nitrite (9.11 g, 121 mmol) was added very slowly as to not create a foamy mess in the reaction flask. After stirring overnight, the material was washed with base, extracted with methylene chloride, and reacidified to form a solid that was then purified using column chromatography (9:1 $CHCl_3$:MeOH) to afford a white solid that had a varying yield of 30-75 percent and was pure by ^{1}H NMR.



19

Tetraazido functionalized estradiol (19) To a clean, dry 40 mL RBF was added estradiol **6** (168 mg, 0.758 mmol), 3,5-diazidobenzoic acid **18** (334 mg, 1.636 mmol), in 15 mL of dry CH_2Cl_2. 2.5 equivalents of DMAP were added as an activator. The mixture was cooled down to zero degrees Celsius using an ice water bath. DCC (297 mg, 1.44 mmol) was dissolved in 5 mL of CH_2Cl_2 and was slowly added to the reaction mixture over a period of an hour. The reaction was allowed to go to completion and was monitored via TLC. After column purification (5:1 PE:EtOAc) **19** was afforded in 75% yield that was pure by ^{1}H NMR.

Generalized dendron synthesis procedure: The dendrons were synthesized using the same conditions as above. An allylated dendron was treated with potassium osmate dehydrate with the necessary equivalents of NMO (1.2 eq per alkene) and allowed to react until it was noticed that there were no more alkenes via NMR. This material was then extracted and used for

the next step. The polyhydroxyl material was then allylated using sodium hydride and allyl bromide and purified using the normal purification steps as outlined in the previous syntheses.

Generalized click procedure: To a RBF was added equal portions of water and methylene chloride. Alkyne dendron (4.5 eq) was added as well as the tetraazidoestradiol (1 eq). CuI (0.4 eq) was added and the reaction was allowed to stir under nitrogen and was carefully monitored by MALDI for completion. Once it appeared the reaction was complete, the crude material was run through a silica gel column to remove copper salts (10% methanol in chloroform). This material was then purified further by running through an SEC column to separate the larger molecular weight materials from the smaller dendrons that were left over. It was noticed on practically all the MALDI's that there was some alkyne coupling occurring, possibly limiting the amount of material able to react with the azides.

Generalized crosslinking procedure: Dendrimer was added to a clean dry 1 L RBF and enough dry, distilled methylene chloride was added to ensure a concentration of 1E-5 M. Grubbs second generation catalyst was added (5 mol % per alkene) and the reaction was allowed to reflux under nitrogen until it was complete via MALDI. After completion, the material was purified by silica gel chromatography (10% methanol in chloroform) to remove any ruthenium salts. It was noted however, that removal of these salts can be very difficult, so alternative methods must be sought in order to remove it more efficiently.

Optimized procedure for hyperbranched polymerization: To a clean dry 3 necked 250 mL round bottom flasked equipped with mechanical stirrer and two septums was added the trimethylolpropane (5.00 g, 37.26 mmol) with 50 mL dry diglyme. Sodium hydride (111 mg, 3.726 mmol) was added and gas evolution was noticed. The reaction was heated to 95°C and glycidol (13.7 g, 186.3 mmol) was added slowly (0.5 mL/hr) via syringe pump. Upon completion of glycidol addition, the reaction was dissolved in methanol and neutralized by filtration through an Amberlite cation exchange column. The polymer was twice precipitated from methanol into acetone and dried at 80°C in vacuo. Polymer would show M+Na or M+K peaks on MALDI with a difference of 74.1 mass units (one glycidol monomer).

Procedure for allylation of hyperbranched polymer

The polyol (1.00 g) was dissolved in a 50 % solution of sodium hydroxide (2 mL). Tetrabutylammonium bromide (2 mg) was added and allyl chloride (6 mL) was added over 16 hours via syringe pump. Upon completion of addition, the reaction was acidified with 10% HCl, and allylated polyol was extracted with ether (2 x 50 mL) and evaporated under reduced pressure to afford a much less viscous yellow liquid than the polyol. Polymer would show M+Na or M+K peaks on MALDI with a difference of 114 mass units (one glycidol monomer + allylation).

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